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Diagnosis of Drowning by Combined Computer-Assisted Histomorphometry of Lungs with Blood Strontium Determination*

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ABSTRACT: The aim of our study was to examine the combined contribution of computer-assisted histomorphometry of lungs with blood strontium (BS) measurement to the diagnosis of drowning in cadavers recovered from fresh water. The study population comprised 116 drowned subjects. The results for this group were compared with those obtained for three non-drowned groups: 22 subjects who died from causes other than asphyxia, 13 subjects who died of asphyxia (strangulation or hanging); and 23 healthy living subjects in whom normal BS level was measured. Samples of water where the bodies had been found were analyzed in order to establish a relation with the BS concentration of the drowned subjects. Histologically, each type of pulmonary lesion (congestion, edema, alveolar macrophages, alveolar hemorrhage, and emphysema aquosum) was evaluated semiquantitatively using a score according to the severity of the pathology. Then, a quantitative histomorphometric study was performed using a computer-assisted image analyzer to measure the length and thickness of the alveolar walls, and the area and density of the alveolar cavities. The mean values of the BS levels in the 116 drowned subjects and of the water strontium concentrations were found to be much higher than in the living individuals. Although the ranges were wide, we found no overlap between values found in drowned subjects and those in non-drowned subjects. Emphysema aquosum and to a lesser extent alveolar hemorrhage were found to be the most significant histologic changes in the drowned and asphyxia groups compared with the nonasphyxia control groups.

KEYWORDS: forensic science, forensic pathology, death, drowning, strontium, lungs, histomorphometry, blood

The diagnosis of drowning is one of the most difficult tasks in forensic pathology, because, among other reasons, there are no specific findings on which to base the diagnosis. The measurement of a substance whose concentration in blood is affected by the penetration of water or ions into the lung has been proposed to confirm the diagnosis. To this end, various analyses have been carried out: blood chloride (Gettler Test) (1), blood iron (2), blood magnesium (3), atrial natriuretic peptide (ANP) (4), diatoms (5,6), and strontium (7). The strontium concentration of cadaver blood has been previously proposed as a possible marker for drowning, but its reliability has long been disputed because blood strontium (BS) levels have been shown to vary enormously, and to overlap, both in different parts of the body and in non-drowned and drowned populations (8–10).

Histological investigations are of primary importance, not only to evidence drowning-related pulmonary changes such as congestion, edema, alveolar macrophages, alveolar hemorrhage, and emphysema aquosum, but also to look for lesions in other organs that may have been a possible cause of death. The above-cited drowning-related pulmonary changes are not specific; moreover, their heterogeneous distribution in the lungs makes it difficult to find strong evidence of drowning. For this reason, quantification of lesions by histomorphometry might provide more objective data than qualitative histology. Moreover, biochemical and histologic methods for the diagnosis of drowning have been evaluated separately, in a small number of cases, but seldom when used together (7–10).

In the present study we examined conjoint contribution of a quantitative computer-assisted method for the histologic examination of the lungs with BS determination to the diagnosis of drowning in cadavers recovered from fresh water.

Methods

Definitions

In the present study the term drowning has been used to define a death due to submersion in fresh water, regardless of the mechanism of death.

We included in our control asphyxia group cases of manual strangulation or throttling—strangulation by ligature and hanging.

Population

Drowned group—The study population comprised 116 subjects, 69 males and 47 females, whose deaths were adjudicated as drowned by a medical examiner. In this group, 70 of the 116 subjects underwent autopsy and BS measurement, and the remaining 46, autopsy, BS measurement and histomorphometry. The circumstances of death were taken into account when known. Because the value of any method for the diagnosis of drowning greatly depends on the time elapsing between death and autopsy, we divided these 46 subjects into two subgroups, one with signs of putrefaction and the other without.

Control groups—The results for the drowned group were compared with those obtained for three non-drowned groups. The first

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of these groups comprised 22 subjects, 14 males and 8 females, who died from causes other than asphyxia. These subjects were divided into two subgroups comprising 12 unputrefied corpses and 10 putrefied. A second control group comprised 13 subjects, 7 males and 6 females, who died of asphyxia. Histomorphometry was performed on these 35 control subjects. Finally, the third control group consisted of 23 living male subjects in whom baseline BS level was measured. These individuals were randomly selected among drivers who had been stopped by the police for possible ''driving under the influence'' and given breath tests. The strontium levels measured in cadaver blood are known to be in excellent agreement with those of living individuals (9).

Methods

Strontium measurement—At autopsy, blood was taken from the heart. In addition to BS determination performed on the 116 drowned persons and 23 living ones, samples of water where the bodies had been found were analyzed in order to establish a relation with the SB content of the drowned subjects. For the measurement of strontium we used an atomic absorption spectrophotometer (Perkin-Elmer, model 2380, Saint-Quentin-en-Yvelines, France) equipped with a standard Perkin-Elmer hollow cathode strontium lamp and an AS-40 autosampler. A 2012 digestor (Trecator, Sweden) was used for mineralization. Distilled deionized water was employed for the preparation of all solutions. Plastic tips for Eppendorf pipettes were rinsed with ultrapure water immediately before use; 15 mL of whole blood were added to 5 mL of 14 N nitric acid in a 100 mL Pyrex glass tube. Whole blood was mineralized using concentrated nitric acid at 200°C during 30 min, before assay. Next, the residue was reconstituted with distilled water and adjusted to 25 mL. A 20 μL volume of unknown sample solution or standard solution sample was introduced into the pyrolitically coated graphite tube, using the autosampler AS-40, and duplicate integrated adsorbance measurements were done.

Sensitivity—The sensitivity, defined as the mass (pg) of strontium required to produce an average absorbance area of 0.0044 A.S., was 1.4 pg.

Detection limit—The detection limit calculated for blood and defined as three times the standard deviation of a matrix blank was 10 μg Sr/L blood.

Linear range—The linearity observed was at least up to 100 μg Sr/L blood. Linearity was evaluated by extending the calibration curve to 0.300 A.S.

The calibration standard solutions were prepared to equalize the concentration of strontium in the sample. The detection limit was 0.02 μg/mL.

Histologic and Histomorphometric Study

Histologic examination of the lungs was performed on the 46 subjects of the drowned group and the 35 subjects of the nondrowned groups. Five lung tissue specimens were obtained from peripheral as well as from central parts of the lungs. The specimens were fixed in 4% formalin solution, and, after embedding in paraffin, two sections of 5 μm thickness were cut from each paraffin block. The sections were stained with H & E and Gordon Sweet for reticulin. Thus, ten slides were examined for each subject. The main lesions observed were as follows: congestion, edema, alveolar macrophages, alveolar hemorrhage, and alveolar wall disruption

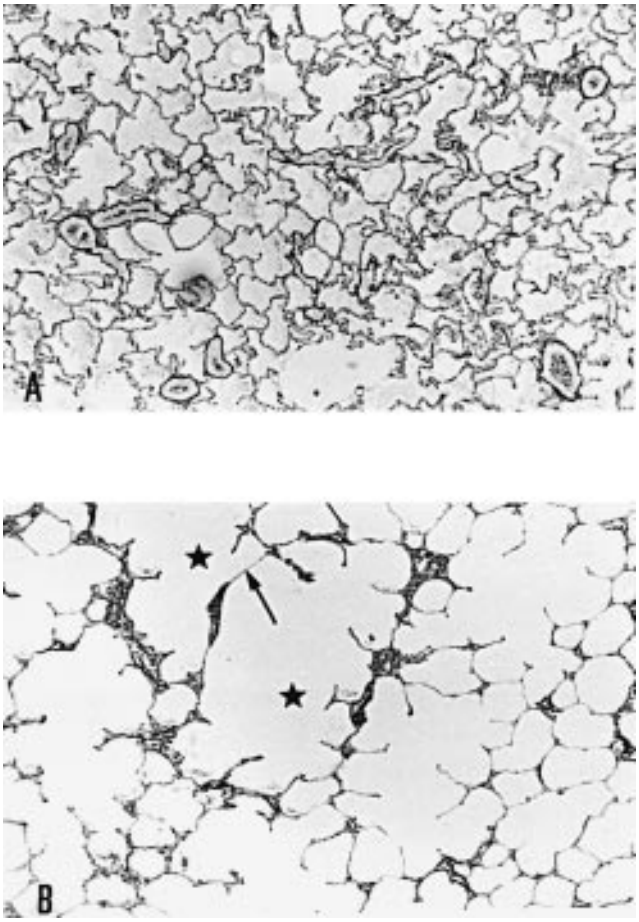

FIG. 1—*Histologic sections of lungs. Panel A: normal. Panel B: ''emphysema aquosum,'' characterized by almost ''empty'' and dilated alveoli (star). The alveolar walls are thin, elongated and often ruptured (arrows). Original magnification: ×40; stain: hematoxylin-eosin.*

(emphysema aquosum) (Fig. 1). Prior to the histomorphometric study, each type of lesion was evaluated semiquantitatively using a score ranging from 0 to 3 according to the severity of the pathology. Grade 0 corresponded to no detectable lesion, grade 1 was characterized by occasional and discrete lesions, grade 2 denoted a limited number of moderate lesions, and grade 3 represented extensive, widespread, and severe lesions. The evaluation was carried out on coded slides under blind conditions. The mean values of the scores obtained from the five regions examined for each subject were calculated for each type of lesion, for each subject and for each group.

After histologic examination of the 405 lung specimens and semiquantitative evaluation of lesions, quantitative histomorphometry was performed on two randomly selected reticulin stained sections for each subject. All histomorphometric measurements were carried out on coded slides under blind conditions. For the histomorphometric study we used a computer-assisted image analyzer. The image analysis system comprised four elements: a light microscope (Nachet, Dijon, France), a black-and-white video camera (Sony, Tokyo, Japan), an image analyzer (NS 15000, Microvision, Evry, France), and a microcomputer (Apple Computer, CA). This microcomputer is connected to the image analyzer, allowing automatic access to all morphological transformations according to a customized program. Each field was digitized in 512 × 512

pixels with 256 levels of gray. Stained areas belonging to large bronchi, pleura and large blood vessels were excluded from data acquisition. Fifteen random microscopic fields were measured at a final magnification of 40 for each subject and the mean values of parameters were calculated. A total of 1215 measurements were performed.

The following parameters were measured: the total area of the alveolar walls, the total length of the alveolar walls, the mean alveolar wall thickness, the number of alveolar spaces per unit area, and the mean area of alveolar spaces. In Fig. 2 displaying the images obtained by the image analyzer at each stage of processing, the points indicate alveolar spaces visualized by the image processor (panel B). Panels C and D show the computerized image of the interstitial tissue from which were measured the total area of alveolar walls and the mean alveolar wall thickness. The computerized tracing of the alveolar wall allowed the measurement of the total length of the alveolar wall. For each parameter, the microcomputer calculated the mean value of the 15 measurements performed for each subject.

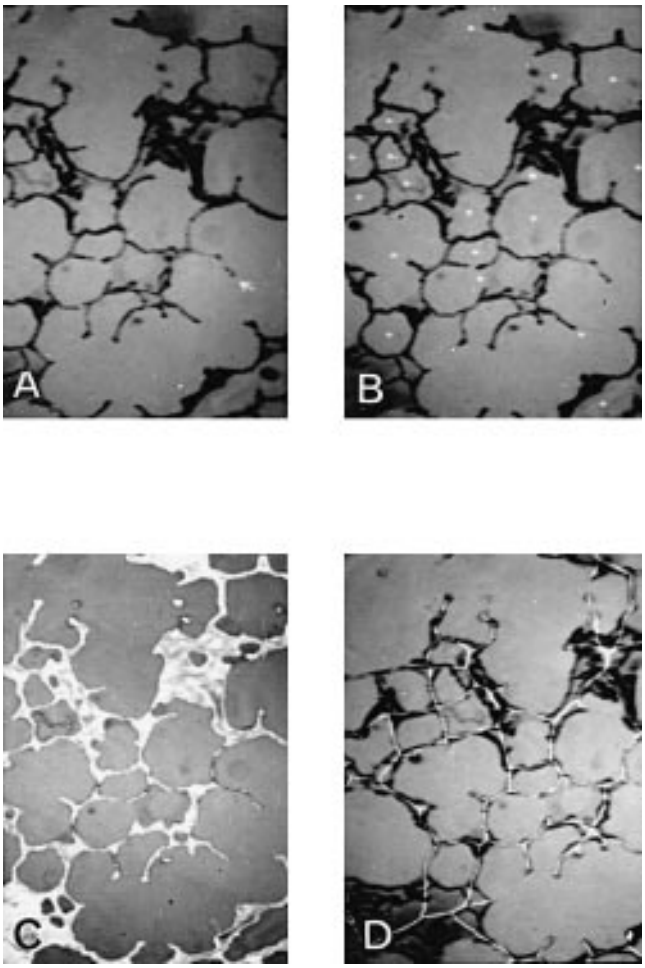

FIG. 2—*Computerized images in 512 × 512 pixels with 256 levels of gray obtained by the image analyzer at four stages of processing. Panel A: initial digitized image obtained after morphological mathematical transformations by the image analyzer. Panel B: The points indicate alveolar spaces visualized by the image processor; Panels C and D show the computerized images of the interstitial tissue. The computerized tracing of the alveolar walls allowed the measurement of the total length and area of the alveolar walls, as well as the number of alveolar spaces per unit area, and the mean area of alveolar spaces.*

Statistical Analysis

For each parameter, mean values obtained in the drowned group and in the control groups were compared using an analysis of variance. A p value <0.05 was considered significant.

Results

Table 1 shows the different sites where the bodies found, and for each site the mean values, median and ranges of the water and blood strontium levels. Normal BS concentrations of living persons were found to be in a relatively narrow range with a mean value of 29 μg/L, median, 32 μg/L (range, 16 to 40). As expected, the mean values of the BS levels in the 116 drowned subjects and of the water strontium concentrations were found to be much higher than in the control group of living individuals, but the ranges were wide. Nevertheless, in the present series, we found no overlap between values found in drowned subjects and those in non-drowned populations. The BS levels were not influenced by age.

Table 2 shows the results of the semiquantitative histologic study. Congestion was found to be slightly but not significantly more marked in the asphyxia group than in the others, a result in accordance with the higher organ weights measured in the asphyxia group. As expected, edema was found to be a nonspecific change. There were fewer alveolar macrophages in the nonputrefied drowned subjects than in the other groups, probably due to a wash-out effect by the water leading to a partial removal of the macrophages from the alveolar cavities. The increase in the number of alveolar macrophages found in the putrefied drowned subjects is likely to be due to a detachment of macrophages and pneumocytes from the alveolar walls during the putrefaction process. Emphysema aquosum and, to a lesser extent, alveolar hemorrhage were found to be the most significant changes in the drowned and asphyxia groups compared with the nonasphyxia control groups.

Table 3 gives the results of the histomorphometric study. In the nonasphyxia control group, parameters were not significantly influenced by the putrefaction process. Regarding the alveolar walls, only their length was significantly reduced in the drowning group compared with the nonasphyxia control groups. The area and thickness of the alveolar walls were found to be similar in the drowned and control groups. The area and number of alveolar spaces were found to be slightly increased and reduced, respectively, but not significantly. Interestingly, parameters obtained in the asphyxia group were found to be significantly modified compared with those obtained in the drowned group. The thickness and area of the walls were found to be increased, and their length decreased. The area of alveolar spaces was larger and the density smaller in the asphyxia group compared to the other groups. The apparent enlargement of the alveolar walls in the asphyxia group was likely to be due to the marked congestion found in the asphyxia group. Of note, persons from the asphyxia group were found to have frequently ingested alcohol or other substances or both for suicidal purposes. This might partly explain the marked congestion found in the asphyxia group.

Discussion

Strontium measurement has been proposed for a long time as a possible marker of drowning, but its diagnostic value has been controversial. The mean strontium concentration in whole blood was found to vary from 16 to 95 μg/L (10), in the plasma from 28 to 44 μg/L and in the serum from 20 to 46 μg/L (10). Some deep subterranean waters contain up to 31,000 μg strontium per

TABLE 1—*Mean values, median and ranges of the water and blood strontium concentrations.*

	Strontium Concentrations			
	Bathtub	Canal	Pond, Lake	River
Water	243; 220; (150–500)	847.8; 977; (125–1035)	49; 400 (2 cases)	397.25; 400 (140–560)
Drowned subjects	140.5; 124 (80–280)	277.7; 210 (80–670)	136.66; 120 (100–160)	150.8; 127.5 (80–320)
Non-drowned subjects	47.75; 45 (40–61)	40 (1 case)	No case	50; 50 (2 cases)

TABLE 2—*Mean scores of lesions observed in the lungs from the study groups.*

Groups	Congestion	Edema	Alveolar Macrophages	Emphysema Aquosum	Alveolar Hemorrhage
Drowning					
No putrefaction	2.37	1.75	1.6	2.5*	1.25*
Putrefaction	2.07	2.50	2.01	2.30*	0.75
Asphyxia	2.60	1.83	1.77	2.85*	1.05*
Controls					
No putrefaction	2.48	2.10	1.96	1.51	0.60
Putrefaction	1.51	2.05	1.48	1.35	0.47

* Indicates a significant difference ($p < 0.05$) between the values obtained in the drowned or asphyxia groups and the control group.

TABLE 3—*Results of the histomorphometric study.*

	Alveolar Walls			Alveolar Cavities	
Groups	Area	Length	Thickness	Area	Density
Drowning					
No Putrefaction	1.18	6.13	2.04	227.63	134.25
Putrefaction	1.08	5.42	2.09	307.37	109.39
Asphyxia	1.41*	4.60*	3.10*	358.56*	86.35*
Controls					
No Putrefaction	1.18	6.56	1.90	199.42	144.90
Putrefaction	1.17	6.48	1.99	261.79	119.57

* Indicates a significant difference ($p < 0.05$) between the values obtained in the drowned or asphyxia groups and the control group.

liter, while in seawater levels of 13,000 μg/kg have been reported (10). Strontium levels in superficial fresh waters vary from 100 μg/L to 10,000 μg/L (10). In drowning, water and strontium-salts reach the blood compartment quickly via the alveolocapillary membrane. Abdallah et al. demonstrated that the strontium concentrations in drowned rabbits were much more elevated than in the control animals dying under anaesthesia (11). Moreover, they asserted that the relevant strontium blood levels were higher in rabbits drowned in seawater than in animals drowned in fresh water. Piette et al. corroborated, albeit with a limited number of cases, the findings of Abdallah et al. on the basis of medicolegal autopsies performed on freshwater drowning cases (9,10). Strontium content in the body fluids has been found to be nonsignificantly effected by resuscitation, haemolysis due to osmotic changes, or early putrefaction (11).

Because cadaver blood is quickly hemolyzed after death we, like others (9), determined strontium in whole blood. The analytical procedure described in the present study is reliable and relatively simple and can thus be employed for specific medicolegal purposes. Furthermore, the strontium levels obtained from cadaver blood have been found in excellent agreement with those of the live subjects (9). The reported average BS concentrations vary from 16 to 95 μg/L total blood (10). Our results are in accordance with these data. Piette et al. found normal BS in whole blood of living persons to be in a relatively narrow range with a mean value of 11.4 ± 0.83 μg/L (10). The values obtained for 22 persons ranged from 5.7 to 15.6 μg/L, with two outliers (20.1 and 22.6 μg/L) (9). In order to establish a relation with the strontium blood content of the drowned person, the strontium content of the water must be determined. Indeed, our data clearly confirm that the strontium concentrations in different sites can vary enormously. In the literature, strontium concentration in some rivers has been found to be similar to that found in blood, but in other rivers, the concentration could be more than ten times higher (10). The putrefaction of the teguments, the postmortem traumatic injuries, and the body damage caused by the flora and fauna might in a substantial manner increase the strontium levels in the body after death. This is due to the mixture of water strontium and blood strontium. In these cases, the reliability of BS determination is questionable, and correlation between BS and other findings are therefore necessary.

Regarding histologic examination of lungs, the lack of specificity of lesions and their heterogeneous distribution make it difficult to ascertain the diagnosis of drowning. Betz et al. investigated whether an estimation of the amount of alveolar macrophages could be used as an indicator of death by drowning in putrefied and non-putrefied lungs (12). They examined the number of alveolar macrophages in lung tissue from 17 cases of freshwater drowning, 22 cases of sudden death and 6 cases of lung emphysema. In cases of drowning, the ratio of ''alveolar macrophages/interstitial tissue'' was reduced in comparison with the other groups, however, without significant differences. These morphometrical results characterizing the ''emphysema aquosum'' with almost ''empty'' and dilated alveoli have been explained by a washout effect of the drowning fluid leading to a partial removal of the macrophages from the alveoli (12). In putrefied lungs, the decay-related destruction of the lung architecture leads to a detachment of macrophages from the alveolar wall, and therefore restricts the value of this parameter. Our results obtained from putrefied lungs showed neither constant values nor a clear tendency to an increase or a decrease with advanced postmortem interval. Therefore, this parameter should be used with caution for the diagnosis of drowning in putrefied corpses.

An important histological finding in the lungs of drowned subjects is the acute dilation of the alveoli with extension, elongation,

and thinning of the septa and compression of the alveolar capillaries. Our histomorphometric data confirmed that these changes are the most significant findings in drowned subjects. The criteria and characteristics for classification presented by Reh, the distinction of typical and atypical drowning, and his division into phases I-IV have not been universally accepted by other investigators (13). In the case of putrefied corpses found in water where there is extensive destruction of pulmonary tissue, histological examination of multiple sections of the lungs can make a valuable diagnostic contribution in visualizing the alveolar reticular fiber structure. Because pulmonary lesions are distributed heterogeneously acute expansion of the alveoli is required to be distributed over large sections of the lungs.

Finally, the fact that our quantitative data show significant differences between the drowned subjects and the asphyxia ones regarding alveolar walls/cavities parameters might be of diagnostic value, but further studies are needed to confirm our findings.

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